RECIPIENT GENE DUPLICATION DURING
GENERALIZED TRANSDUCTION

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ABSTRACT

An Hfr13 $\Delta^{proA-lac}$ *deletion* recipient, $-\Delta^{proA-lac}$-F-*purE*+-, has been util-
ized in a study of the origins of duplications formed during chromosome frag-
ment integration. Among the Pro‾Lac+ transductants, some have duplications
spanning the F locus. These transductants are, or segregate, strains with F'
episomes carrying genes of the duplication. Some of the duplications include
purE+, a gene which is not coinherited with *lac*+ during bacteriophage P1-
mediated transduction. Thus recipient genes have been duplicated during
recombinant formation. Crossing-over models including replication steps pro-
vide a basis for explaining the duplication process.

HETEROZYGOTES are sometimes formed during generalized transduction of
bacterial genes (LENNOX 1955; MARKOVITZ 1964). Their unstable genetic
elements are now recognized to be components of *cis*-oriented chromosomal dup-
lications (HILL *et al.* 1969). This study is concerned with the origins of genes in
duplications so formed. In a previous study, *lac*+ transduction from *Escherichia
coli proA*+ *lac*+ to recipients with *proA-lac* deletions was characterized
(STODOLSKY, RAE and MULLENBACH 1972). This system provides an absolute
selection for partial diploids, because the absorbed *lac*+ fragments are too short to
be integrated by a replacement of the deletion. Transductants have genetically
unstable *lac*+ units, designated *lacV*, which are integrated in tandem with the
deletion. The formation of their recombinant chromosomes can be depicted as
proceeding through some sequence of crossing over and fusion. Plausible candi-
dates for the critical stablizing event of fusion (end-to-end joining of genetically
heterologous DNA's) are: a base pairing of "accidentally" complementary DNA
ends; the priming of DNA synthesis (GOULIAN 1968; STODOLSKY 1973a); and
ligation (SGARAMELLA, VAN DE SANDE and KHORANA 1970).

This study was motivated by the following observations. The $\Delta^{proA-lac}$/*lacV*
heterozygotes are diverse in the stability of their *lac*+ genes, ranging from quasi-
stable types to some which are almost too unstable to maintain even under selec-
tive conditions. It is plausible to attribute differences in heterozygote stability to
the length of their duplicated gene segments. The longer a duplication, the
higher the probability (per generation) that crossing over within units of the
duplication will occur; if the crossover is between distinct components of the
duplication, a haploid segregant is formed. Accepting this working interpreta-

tion, it seemed possible that the more unstable heterozygotes might have duplications longer than the chromosome of the ancestrol *lac* fragment. That is, recipient genes which were not represented in the absorbed *lac*+ fragment might be included in the duplication of the recombinant $\Delta^{proA-lac}/lacV$ chromosome. To simply test this suspicion a novel trick has been employed in this study. The difficult task of *lacV* gene content analysis has been replaced by a simpler but equivalent F'*lac* gene content analysis. It appears that duplications of recipient genes do occur during the generalized transduction. A molecular basis for the duplication process is offered. A preliminary report of the results has been presented (STODOLSKY 1972).

TABLE 1

E. coli *K12 strains*

Strain	Relevant characters	Source
χ316	F⁻ prototroph	R. CURTISS III
χ316	F⁻Δ *proA-lac* deletion	R. CURTISS III, from χ316
W3747†	*met⁻*/F'13 *argF⁺ lac⁺ proC⁺ lon⁺(capR)⁺ purE⁺*	W. EPSTEIN
SC1500	F⁻Δ*111 (proA-argF-lac deletion X111)* *his strA metB argI*	W. EPSTEIN
SC1401	Δ*proA-lac*/F'13 *lac⁺*	W3747 × χ354
SC1402	Hfr13 Δ*proA-lac*	segregant of SC1401
SC1405	F⁻Δ*proA-lac strA‡*	spontaneous mutant of χ354
SC0622	F⁻Δ*111 recA1*	STODOLSKY *et al.* (1972).
MC100	*leu lacY proC purE galK trp strA*	A. MARKOVITZ, χ156 of R. CURTISS III
MC102	*leu lacY capR9 purE galK trp strA*	A. MARKOVITZ
KL1699	HfrKL16 *recA1*	W. EPSTEIN
SC0701	HfrKL16 *recA1 mal* (λ^r)	Spontaneous mutant of KL1699, resistant to bacteriophage λ and Mal⁻
SC1416	Δ*proA-lac*/F'*lac⁺ proC⁺ lon⁺ purE⁺* (F'6)	Lac⁺ transductant of SC1402
SC1426	Δ*proA-lac thyA*/F'6	Trimethoprim-resistant§ mutant of SC1416
SC1427	Δ*proA-lac recA1*/F'6	SC0701 × SC1416¶
SC8306	Hfr13 Δ*proA-lac purE*	SC1402 × MC102

* χ354 was initially given a *proA⁺ proB-lac* designation by R. CURTISS III. However, I have found that χ354 has an amber suppressor, which probably led to its mis-characterization following introduction of *pro* amber alleles. Recent deletion mapping and complementation tests demonstrate that: there is no essential "*proB⁺*" marker between *argF* and *lac* (contrary to the current *E. coli* map); χ354 lacks *proA*; the initial *proB* and *proA* distinctions (based solely on conjugal cross data) are not reliable predictors of complementation behavior (ACKERMAN and EPSTEIN, personal communication). Complementing *pro* alleles (exclusive of *proC*) are very tightly linked (CONDAMINE 1971; STODOLSKY 1973b). For these reasons *proB* is not utilized herein, and *proA* is utilized only as a general chromosomal locus designation.

† *Lon* and *capR* are alternative designations for a locus pleiotropic for control of radiation sensitivity and capsular polysacharide synthesis.

‡ *StrA* strains are resistant to 100 μg/ml of streptomycin (*str*^R).

§ Selected on minimal agar containing 10 μg/ml Trimethoprim (Burroughs, Wellcome and Co.) and 200 μg/ml thymine.

¶ SC1426 was converted into an F⁻ phenocopy by growth at 25°, prior to a 30-minute mating. SC1427 was identified as an exconjugant with sensitivity to ultraviolet light, comparable to SC0701 *recA1 mal⁻*.

MATERIALS AND METHODS

Media—Synthetic and broth media have been described (STODOLSKY, RAE and MULLENBACH 1972). The minimal media do not contain Ca++, which is required for bacteriophage P1 absorbtion. Thus P1 does not propogate on the transduction plates. On EMB lactose indicator agar, Lac⁻ colonies or sectors are pink, while lactose catabolizing bacteria stain purple to black. Sectors are most readily resolved after 18–24 hrs at 37°.

Bacteriophage—A rapidly absorbing (*g* character) prophage repressor negative P1 mutant, P1*cl g*, was obtained from N. FRANKLIN and used in all transductions.

Bacteria—*E. coli* K12 strains utilized are described in Table 1 and marker loci are depicted in Figure 1. *Shigella dysenteriea Sh* was utilized for P1 titration.

Strain SC8306 $\Delta^{proA-lac}$ *purE strA* was constructed in the following way. SC1402 *Hfr13* $\Delta^{proA-lac}$ *leu+ cap+ pur+ trp+ str*^{S} was mated with MC102 *leu⁻ layY capR9 purE trp⁻ strA*. Conjugal pair disruptions were performed at 30, 60, 90 and 120 minutes. The treated samples were diluted 1000-fold and grown for an hour more to permit the segregation of Str^R recombinants from sensitive *strA/str+* exconjugants. Leu+ Trp+ Str^R-resistant recombinants were selected on minimal agar plates which were permissive for other characters. Cap+ colonies were recognized by their mucoid character. Pro⁻ Pur⁻ colonies were detected by replica plating to suitable minimal agars. The desired recombinant could most simply have arisen through crossovers which integrated a *leu+* $\Delta^{proA-lac}$-*F-cap+* segment of the SC1402 chromosome into the MC102 chromosome, with the recipient *trp⁻* allele being replaced by other crossover events. Pro⁻ recombinants were only obtained from the sample which was disrupted 120 minutes after mating was initiated. This observation shows that $\Delta^{proA-lac}$ is a late marker of SC1402, and justifies (together with the fact that *proC+* is transferred early to MC100 *proC strA*) the $\Delta^{proA-lac}$-*F-proC+* linkage assignment for SC1402.

Transduction—P1*cl g* was absorbed for 30 minutes at 25° to bacteria grown in L broth. Infected bacteria were washed in saline by centrifugation and plated on minimal agars.

Conjugal crosses—Crosses in broth (STODOLSKY, RAE and MULLENBACH 1972) and matings by replica plating (PITTARD 1965) have been described previously.

Purifications—Some P1*cl g* does survive during incubation on the selection plates, and is carried along during transfer of transductant colonies. For this reason it is necessary to disperse
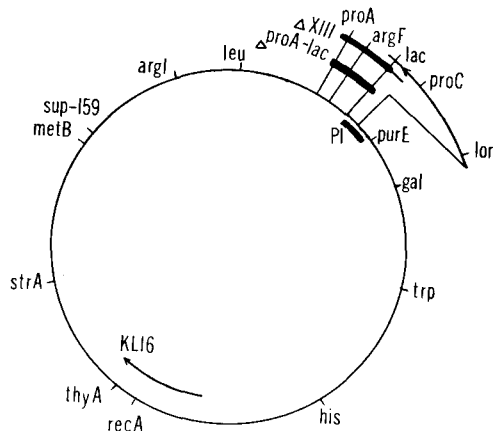

FIGURE 1.—The *E. coli* map adapted from TAYLOR and TROTTER (1972). See Table 1 footnotes for reasons for *proB* omission. The bar designated P1 corresponds to the 35μ length of the bacteriophage P1 chromosome drawn to the same scale as the 1100μ *E. coli* chromosome. It is not known if the *proA-lac deletion XIII* includes the F locus of Hfr13 strains. Justification for placing the origin of Hfr13 between *lac* and *proC* is presented in the text.

transductants in (Ca^{++}-free) saline to avoid P1cl g infections. New strains were purified by at least two subcultures on selection or indicator agars.

The experimental design: A primary problem in undertaking this study was the design of a simple method for determining the extent of duplications. This problem is trivial when a duplication is heterozygous for many of its genes. It then suffices to identify the various segregant types. But when it is suspected that part of a duplication may be homozygous (being derived only from recipient genes) a more general method is necessary. The following stratagem was motivated by PITTARD's (1965) observation that F excision from an Hfr chromosome is stimulated when recombination occurs around the origin of the intergrated F factor.

Consider a duplication in which F is within only one of the genetic repeats (the duplication is "heterozygous" for F). Such duplications will sometimes be formed in transduction during lac^+proC^+ fragment addition to the $\Delta^{proA-lac}$-F-$proC^+$ chromosome of an Hfr13 recipient (Figure 2). The transductant is equivalent to a $\Delta^{proA-lac}proC^+$/F′ lac^+ $proC^+$ heterozygote, except that F′ will be in the integrated state. Episomes carrying lac^+ and the recombinant's duplicated genes will sometimes be excised by legitimate (gene-linkage-conserving) crossovers (CAMPBELL 1962).

In the case of an F′ formed within an Hfr13 population, lac^+ is a distal marker of the episome (Figure 1). Genes transferred from the F′ population with lac^+ in a short mating are on the episome and can be detected through transfers to suit-
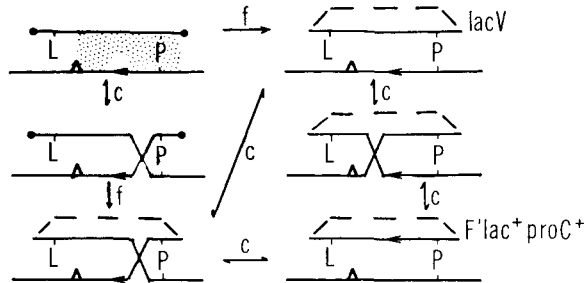

FIGURE 2.—Integration of a lac^+ (L)-$proC^+$ (P) chromosome fragments into an Hfr13 $\Delta^{proA-lac}$ *deletion* (Δ) chromosome, and possible rearrangements of the recombinant Hfr13 $\Delta^{proA-lac}$/$lacV$ chromosome. Genetic joints formed by fusion (f) are represented as dashed lines. Legitimate crossovers are designated by (c), and ← defines the F locus and the direction of conjugal chromosome transfer. At the upper left, homologous segments of the fragment and chromosome are aligned. Integration might be accomplished by a crossover followed by fusion, as depicted on the left. Alternatively fusion of the fragment ends could produce a circular $lacV$ genote, and integration could then proceed through a single reciprocal crossing over. Intrachromosomal recombination within the recombinant Hfr13 $\Delta^{proA-lac}$/$lacV$ chromosome can result in the excision of a $lacV$ genote. If the integrating crossover and the excising crossover are on opposite sides of F, an autonomously replicating F′lac^+-$proC^-$ episome is produced (lower right).

able multiply auxotrophic recipients. Thus the characterization of F'*lacs* of transductant populations provides a simple and rapid method for assaying the gene content of the original duplication.

An alternative but more laborious procedure is the identification of the repeat point or joint (*jnt*) of the duplication through transduction crosses (HILL, SCHIFFER and BERG 1969).

Construction of the recipient: To construct the required Hfr recipient for transduction the F'13*lac* episome of W3747 *metB*/F'13*lac*$^+$ *proC*$^+$ was introduced into x354 $\Delta^{proA-lac}$ and SC1401 $\Delta^{proA-lac}$/F'13 was selected. To obtain the desired Hfr segregant, the F' population was propagated at 37° in L broth. Under these conditions infection of F$^-\Delta^{proA-lac}$ segregants with F'13 is rampant. Consequently the Lac$^-$ segregants which accumulate are *Hfr13* $\Delta^{proA-lac}$ strains. F'13 could not propagate as an episome in these segregants, even if absorbed, because of competition for F membrane maintenance sites. SC1402 Hfr13 $\Delta^{proA-lac}$ was picked as a Lac$^-$ segregant of SC1401 $\Delta^{proA-lac}$/F'13. It transfers *proC*$^+$ as an early marker. It transfers $\Delta^{proA-lac}$ as a late marker, as found during the construction of SC8306 *Hfr13* $\Delta^{proA-lac}$ *purE* *str*^{R} (see MATERIALS AND METHODS).

Transductions: A prophage repressor-defective bacteriophage P1, P1*cl g*, was used as the vector in all transductions and m.o.i. of 0.3 were employed. P1*dlac* and P1*dpro* lysogens are sometimes formed in recipients of P1*cl*$^+$ and transducing particles (LURIA, ADAMS and TING 1960; RAE and STODOLSKY 1973; STODOLSKY 1973b). The use of a P1*cl* vector has barred their formation in this study.

Gross characteristics of the transduction system are presented in Table 2. Lac$^+$ transduction of x354 $\Delta^{proA-lac}$ is about 10-fold less efficient than transduction of the x474 *lacY* recipient. In the latter strain *lac*$^+$ integration can proceed by a simple replacement of the *lacY* allele. A comparison of *lac*$^+$ transduction to x354 F$^-$ $\Delta^{proA-lac}$ and SC1402 *Hfr13* $\Delta^{proA-lac}$ suggests that the presence of F in the chromosomal region in which the *lac*$^+$ fragments must be integrated does somewhat diminish the efficiency of *lac*$^+$ integration by addition. *Pro*$^+$ gene addition to these two strains proceeds at identical frequencies.

F' strain formation in transductants of SC1402 Hfr13 $\Delta^{proA-lac}$str^S: To simply identify F' strains among transductants, a qualitative test distinguishing early and late marker transfer was employed. Markers transferred after *str*^{S} from

TABLE 2

Transduction of lac$^+$ *and* proA$^+$ *from* x316 pro$^+$lac$^+$pur$^+$ *to* $\Delta^{proA-lac}$ *recipients*

Recipient	Lac$^+$	Transductants per infectious virion for: Pro$^+$	Pur$^+$	Lac$^+$Pro$^+$	Lac$^+$Pur$^+$
x354 F$^-$ $\Delta^{proA-lac}$	9×10^{-8}	2×10^{-7}	—	—	—
SC1402 *Hfr13* $\Delta^{proA-lac}$	3×10^{-8}	2×10^{-7}	—	—	—
SC8306 *Hfr13* $\Delta^{proA-lac}$ *purE*	*2×10^{-8}	—	2×10^{-6}	—	*<3×10^{-9}
x474 F$^-$ *proA lacY purE*	10^{-6}	10^{-6}	3×10^{-6}	*<3×10^{-9}	*<3×10^{-9}

* Transductants and controls (10^{10} of each) were plated on 2 ft. square plastic dishes, to avoid exhaustive catabolism of supplements by concentrated bacteria.

Hfr13 str^S donors rarely appear in str^R exconjugant lines. Str^S is dominant in new str^R/str^S exconjugants, and recipients of markers distal to str^S are killed when the selection is applied prior to haploidization. Lac^+ or pro^+ genes added to the Hfr13 $\Delta^{proA-lac}$ chromosome next to the deletion would be transferred as late markers. However F′ segregants of transductants would transfer out F′ independently of the host str^S chromosome, and str^R/F′ exconjugants could thereby be obtained.

Transductants were characterized in parallel using a plate mating technique (PITTARD 1965). Pro$^+$ and Lac$^+$ transductants were spread in patches on the original selection agars and replicas of these master plates were used in conjugal crosses. Crosses were initiated by replicating transductant patches onto SC1504 $\Delta^{proA-lac}str^R$ recipients on streptomycin-containing agar. SC1401 $\Delta^{proA-lac}$/F′13 lac^+ served as an F′ control.

Of 19 Lac$^+$ transductants 10 were identified as good lac^+ donors to SC1500 $\Delta 111$ his^-met^-argI str^R. Their Lac$^+$ exconjugants were subcloned on EMB lactose agar and replicated onto lawns of SC0622 $\Delta 111$ $recAI$ his^+ met^+ $argI^+$ on lactose proline agar. Auxotrophies of the donor served as the counterselection. More than 50% of each population of subcloned exconjugants transferred lac^+ to the recombination-deficient recipients. The successive transfer of lac^+ from the transductants to SC1500 and SC0622 establishes that the transductants have fertile episomes.

If the proposed explanation of F′lac formation within the transductants (Figure 2) is valid, then F′pro strains must not be present among Pro$^+$ transductants of SC1402 $Hfr13$ $\Delta^{proA-lac}$. The lack of coinheritance of lac^+ with pro^+ (Table 2) shows that the $proV$ elements in recombinant chromosomes ($-proV-\Delta^{proA-lac}-F-proC^+$) do not genetically span the deletion. Hense the $proV$ elements do not span the F locus as well, and F′ pro formation cannot proceed through the legitimate excision scheme of Figure 2. Using the plate mating technique, 20 Pro^+ transductants were crossed with SC1405 $\Delta^{proA-lac}str^A$. None of the 20 transductants are early pro^+ donors and therefore do not contain F′pro^+ episomes.

The lack of F′pro formation in the Pro$^+$ transductants and the frequent F′lac formation in the Lac$^+$ transductants provides strong support for the F′ genesis scheme of Figure 2.

To justify the use of F′lac's to determine the gene content of a transductant's duplication, it was first essential to show that the same F′lac is formed in distinct lac^+ excisions from the chromosome of a particular $Hfr13$ $\Delta^{proA-lacA}lacV$ transductant. That is, it is *conceivable* that the recombinations mediating F′lac^+ excisions might more commonly be genetically aberrant than genetically legitimate. Then distinct F′lacs would arise within a single $Hfr13$ $\Delta^{proA-lac}$/$lacV$ transductant line, and F′lac analysis could not be substituted for $lacV$ gene content analysis. To examine this possibility, strains with lac^+ still in the integrated state were sought among the 10 transductant populations which yielded positive results in the patch tests. Six transductants appeared to be predominantly in the F′ state. They were relatively stable for lac^+ and poor conjugal trp^+ donors. In populations of each of the remaining four transductants, two colony types could

TABLE 3

Conjugal transfer from Lac$^+$ *transductants of SC1402* Hfr13 $\Delta^{proA-lac}$ *to MC100* lacY trp str^R

Donor	Exconjugants per donor for*	
	Lac$^+$	Trp$^+$
SC1402 Hfr13 $\Delta^{proA-lac}$	—	0.2
Transductant 4†		
Hfr13 $\Delta^{proA-lac}$/ *lacV*	0.3	0.1
$\Delta^{proA-lac}$/F'*lac*+*proC*+	0.8	0.006
SC1416 $\Delta^{proA-lac}$ *rec*+/F'6 *lac*+-*purE*+	0.7	0.02
SC1427 $\Delta^{proA-lac}$ *recA1*/F'6 *lac*+-*purE*+	0.5‡	$< 10^{-4}$

* Conjugal pairs were diluted into saline, after 40 minutes at 37° and vortexed (Vortex Junior Mixer) for one minute before plating.
† Transductants 5, 16, and 18 gave similar results.
‡ Lac$^+$ exconjugants were Pur$^+$.

be distinguished on EMB lactose indicator agar: dark colonies which rarely exhibited a Lac$^-$ sector, and more lightly staining and more frequently variegated Lac$^+$ colonies. Variegated colony-forming types segregated the dark colony-forming ones. The variegated to dark colony transition probably reflects the transition of *lac*$^+$ from an integrated state in *Hfr13* $\Delta^{proA-lac}$/*lacV* transductants to an autonomous state in $\Delta^{proA-lac}$/F'*lac* segregants (Figure 2); variegated colonies (one tested for each transductant) were more effective conjugal *trp*$^+$ donors than their dark segregants (Table 3). To determine if the F' strains derived from a single more unstable predecessor were identical, five independent segregants from transductant #4 were compared by the tests described below. Each contained a F' *lac*$^+$ *proC*$^+$ episome. This is the longest episome present among the transductants for which an *Hfr* $\Delta^{proA-lac}$/*lacV* predecessor was detected. Since identical episomes are being formed within a single *Hfr13* $\Delta^{proA-lac}$/*lacV* transductant line, a substitution of F'*lac* gene content analysis for *lacV* gene content analysis is acceptable.

F'lac genes: Exconjugants formed through F'*lac* transfer were characterized to establish the presence of markers other than *lac*$^+$ on the episomes formed in Lac$^+$ transductants of SC1402 *Hfr13* $\Delta^{proA-lac}$ *pur*$^+$. SC1500 $\Delta 111$ *argI*$^-$str^R lacks both *E. coli* K12 genes encoding ornithine transcarbamylase activities; it is *argI*$^-$ and lacks the *argF* gene which is included within the *deletion X111*. The F'*lac* containing exconjugants of SC1500 are Arg$^-$. Thus the counter-clockwise "end" of the episomes is between *argF* and *lac* loci.

Other markers of the episomes were determined using two types of donors with consistent results. The F' type transductants were used as donors. Also $\Delta 111$ *recA1*/F'*lac* exconjugants of SC0622 $\Delta 111$ *recA1* (produced as described above) were used as donors. With *recA1* strains as donors, mobilization of chromosomal markers by crossing over is insignificant. To score for the presence of *proC*$^+$, *purE*$^+$, and *gal*$^+$ genes on the episomes, Lac$^+$ Str^R exconjugants of MC100 *lacY proC purE galK str*^{R} were selected and replicated to suitable minimal media. To score for the presence of *lon*$^+$ *(cap*$^+$*)* on episomes, Lac$^+$Str^R exconjugants of MC102 *lacZ capR9 str*^{R} were selected and subcloned. *CapR9* strains exude capsu-

TABLE 4

Markers of episomes formed in Lac+ transductants of SC1402 Hfr13 Δ^proA-lac pur+

Transductant number	argF+	lac+	Marker proC+	lon+	purE+	gal+
6, 9	. .	+	+	+	+	. .
10	. .	+	+	+	. .	. .
4, 12, 14	. .	+	+	. .	. .	. .
5, 13, 16, 18	. .	+	. .	. .	. .	. .

The donor for transduction was χ316 F⁻arg+lac+pro+lon+pur+gal+.
For transductants 4, 5, 16 and 18, Hfr forms were detected during the purification.

lar polysaccharide which confers a mucoid appearance to their colonies at 37°, while their *capR9*/F′*lac+cap+* derivatives are opaque and compact (MARKOVITZ 1964). The episomes comprise a genetically overlapping family (Table 4). None carry *galK+*. *PurE+* is carried on F′6 and F″9 episomes from transductants SC1416 and SC1419, respectively.

*PurE+ genes of the F′*lac+proC+lon+purE+ *episomes are almost certainly derived from the recipient line*: Some P1 virions might cotransport *lac+* and *purE+*, as the P1 virion chromosome and the *lac+–lon+–purE+* chromosome segment are about the same length (Figure 1). But coinheritance of *lac+* and *purE+* during P1 transduction cannot be detected (Table 2). In fact there is only 0.1% coinheritance of *lon+* with *lac+* during transduction (MARKOVITZ 1964), and the *lac+–lon+* segment is only about half the length of the *lac+–lon+–purE+* segment.

The genotype of SC1416: If the host chromosome of SC1416 Δ^proA–lac/F′6 retains *purE+*, the *purE+* gene was in some way duplicated in the transductant line. A proposal for a genetically legitimate duplication process is developed in the DISCUSSION. However, the following alternative must also be considered. There is only one *purE+* gene in the transductant, and the host Hfr13 Δ^proA–lac *purE+* chromosome in some way suffered a *purE* deletion during the formation of the F′6 *lac+ purE+* episome.

To test the alternative, it suffices to determine the Pur character of F⁻ segregants of SC1416. Each of ten independently isolated Lac⁻ segregants of SC1416 was found to be Pur+. However, it must be demonstrated that such segregants do arise without crossing over with the episome. The qualification is essential. If the hypothetical *purE+* region deleted from the chromosome and transferred to the episome contains genes essential for viability, then only Lac⁻ segregants which had recovered *purE+* by crossing over prior to episome loss could be obtained. To block crossing over prior to F′6 loss a *recA1* defect was *introduced into* SC1416 in two steps. First SC1426 *mal+ thyA*/F′6 was selected as a spontaneous mutant of SC1416 *mal+ thy+*/F′6. It was confirmed that SC1416 still yields Lac⁻ Pur+ segregants. Then SC1416 was converted to an F⁻ phenocopy by growth at 25°. *Thy+–recA1* chromosome segments were *introduced* through a short conjugal cross with SC0701 *thy+ recA1 mal⁻* (see Table 1). Mal+ Thy+

exconjugants were selected and Rec⁻ Lac⁺ types were purified. These exconjugants were the desired Rec⁻ derivatives of the original SC1416 $\Delta^{proA-lac}rec^+$ strain.

For the SC1427 $\Delta^{proA-lac}recA1$ trp^+/F'6 derivative, it is shown that there is little conjugal mobilization of the host trp^+ gene (Table 3). This lack of chromosomal mobilization confirms that there is litttle crossing over between episome and chromosome occurring in the Rec⁻ background. For each of ten distinct Rec⁻ exconjugants of SC1416 $\Delta^{proA-lac}$/F'6, ten Lac⁻ segregants were isolated. All Lac⁻ segregants were Pur⁺. Thus it can be concluded that the $\Delta^{proA-lac}recA1$ chromosomes do encode $purE^+$.

It is conceivable that there was a $purE^+$ deletion in the SC1416 $\Delta^{proA-lac}$/F'6 transductant line, which was in some way eliminated before the introduction of the $recA1$ alleles. However the simplest interpretation of the results is that the host chromosome of the SC1416 transductant line never lost its $purE^+$ gene.

Other transductants: Among the lac^+ transductants which are not efficient and early lac^+ gene donors, three types have been distinguished. Two transductants transfer lac^+ by conjugation after 90 minutes. I presume that $lacV$ is integrated between F and $\Delta^{proA-lac}$ loci, and that $lacV$ does not genetically overlap the F locus. It appears that one transductant is physiologicaally unfit and/or that the heterozygous genotype is exceedingly unstable. Even colonies grown on minimal lactose proline agar (on which proline may be catabolized as a carbon source) contain more than 90% Lac⁻ segregants. This transductant has been set aside for a separate characterization.

The remaining six transductants are all more unstable than the transductants rapidly segregating F' strains. While F⁻ segregants are readily obtained by picking Lac⁻ sectors of the F' strains, F⁻ segregants have not been found among the Lac⁻ segregants of these six transductants. In crosses with SC1500 $\Delta 111$ str^R, F'lac^+-carrying exconjugants are formed, but with only 1–10% the efficiency of the crosses with $\Delta^{proA-lac}$/F'lac strains. The longest episome so isolated carries lac^+ and $proC^+$. A tentative explanation of these characteristics is developed in the DISCUSSION.

DISCUSSION

Heterozygote lines produced through lac^+ transduction of an $Hfr13$ $\Delta^{proA-lac}$ recipient have been analyzed in this study. Some transductants segregate F' strains. It is plausible that the segregation event is a legitimate crossover within a recombinant Hfr13 $\Delta^{proA-lac}/lacV$ chromosome, which yields companion F'lac and F⁻ $\Delta^{proA-lac}$ chromosomal products (Figure 2). The $purE^+$ gene is not cotransduced with lac^+. But two of the ten F' strains carry $purE^+$ on their episomes (Table 4). Evidence that $purE^+$ is also encoded in the host chromosome has been sought and obtained for one of these exceptional heterozygotes. Hence the duplicated $purE^+$ genes of the transductant both appear to be derived from the recipient line. This is the most intriguing observation of this study. How did the recipient gene duplication occur?

A reasonable possibility is that the partial chromosomal replication accompanied an initial crossing-over step in fragment integration. A structure suitable
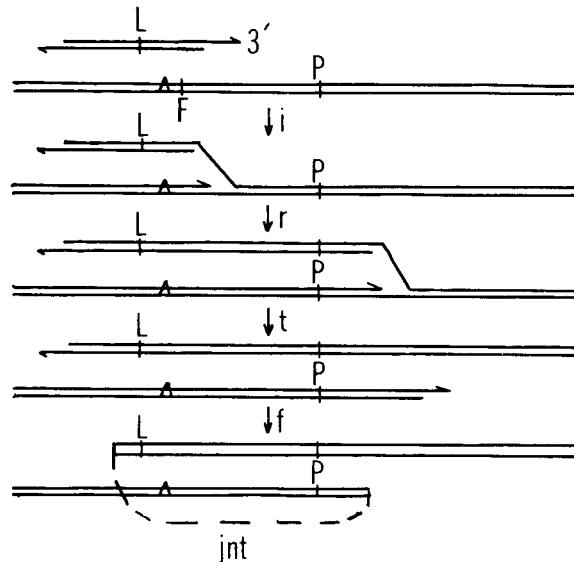
FIGURE 3.—A model for *purE* gene duplication during *lac+* fragment integration. A single crossover is initiated (i) by an attack of the *lac+* (L) fragment strand with a 3′ terminus at a homologous chromosomal locus to the right of the $\Delta^{proA-lac}$ deletion (Δ) and the F locus (F). Ligation of the fragment strand with a recipient chromosomal strand completes the formation of a fork, from which some type of DNA replication (r) proceeds. Unknown factors control the extent of the replicative process which proceeds through *purE* (P) for the case depicted. A breakage of the previously continuous recipient chromosomal strand terminates (t) the replication step. Chromosome continuity is established by fusion (f), with the fusion joint (*jnt*) depicted as a dotted line.

for the initiation of some type of chromosomal replication could be formed as a strand of a transduced fragment requires continuity with a homologous recipient chromosomal strand (Figure 3). If replication proceeds, the fragment branch of the fork is genetically elongated. Thus when integration is completed by replication fork breakage and a fusion of chromosome ends, the resulting duplication can include recipient genes which were not represented on the initially absorbed fragment. The elongation step could proceed whether a 3′ or 5′ strand of the fragment acquires continuity with the chromosome initially. However, I have argued that fragment ends generally acquire single-stranded 3′ terminii in recombination-proficient bacteria, and that the priming of synthesis from a 3′ terminus on a heterologous template strand is the critical stabilizing step in some fusion events (STODOLSKY 1973a). A single-stranded 3′ terminus is specified in Figure 3 only to maintain compatibility with these ideas.

The length of the proposed replication step cannot be estimated from data of this report for at least three reasons. The portion of the duplication derived directly from the absorbed *lac+* fragments is not known. Some duplicated genes may be degraded prior to the establishment of the fusion joint. The rate of degradation of the *lac+* fragment end remaining exposed during replication is not known; if DNA end degradation is rapid, then the selection for transductants

retaining lac^+ would also constitute a selection for recombinants formed through shorter replication steps. A minimal estimate of the length of the longest replication step detected can be made. There is only about 0.1% coinheritance of lac and lon during transduction (MARKOVITZ 1964). It is reasonable to assume, therefore, that the $lon^+–purE^+$ segments of the two $\Delta^{proA–lac}proC^+lon^+ purE^+/F'lac^+ proC^+ lon^+ purE^+$ transductants are derived from the recipient chromosome. The $lon^+–purE^+$ chromosome segment is about 18 microns long.

Six of the nineteeen transductants analyzed are unusual in that their Lac^- segregants are predominantly or exclusively like the Hfr13 $\Delta^{proA–lac}$ recipient for transduction, and yet they are donors of F′ episomes. As a tentative explanation of these characteristics, I suggest that the transductants are double males with F′ episomes integrated into the host chromosomes. For instance, a strain with $\Delta^{pro–lac}–F–proC^+–jnt–lac^+–F–proC^+–lon^+$ gene linkages would correspond to a Hfr13 $\Delta^{proA–lac} proC^+ lon^+$ host with an integrated $F'lac^+ proC^+$ episome. Its Lac^- segregants could only be Hfrs. During conjugation the proximal segment transferred would sometimes include $transfer\ origin–proC^+–jnt–lac^+–F–proC^+–lon$. A segment of this type could give rise to a circular $F'lac^+ procC^+$ episome in the recipient through intramolecular crossing over.

There is a trivial course of events through which a double male transductant could be formed. In a binucleate Hfr13 $\Delta^{proA–lac}$ recipient, all steps in lac^+ fragment integration and $F'lac$ excision (Figure 2) could proceed in a single generation. The cell then contains the $F'lac$, its companion recombinant $F–\Delta^{proA–lac}$ chromosome, and the Hfr13 $\Delta^{pro–lac}$ chromosome which did not participate in the recombinant formation. Thus upon cell division the $F'lac$ genote could segregate with the Hfr13 $\Delta^{proA–lac}$ chromosome. Though the $F'lac$ genote could not replicate autonomously in the Hfr host, it could be integrated into the host $\Delta^{proA–lac}–F–proC^+$ chromosome, yielding a double male recombinant. A more exotic mode of origin would be the following. During lac^+ fragment integration, the replicative step in crossing over is initiated between $\Delta^{proA–lac}$ and F loci. The replication traverses the F factor, and the duplicated F genes are conserved during a subsequent chromosome breakage and fusion. In this case the recombinant product is a double male.

With the selection system utilized in this study only heterozygous Lac^+ transductants can be obtained. But heterozygotes are also formed with donor and recipient are essentially isogenic and without barriers to the formation of haploid recombinants. In the initial analysis of transduction mediated by P1, it was observed that transductant colonies were frequently a mixture of haploids (LENNOX 1955). It now seems plausible that the genetically diverse haploids were segregants of an ancestral heterozygous recombinant. Using a transduction system for which persisting heterozygotes within a clone are readily detected, MARKOVITZ (1964) found that about 30% of the transductants for the $proC–lon$ region are heterozygotes. However the frequency of heterozygote formation during transduction is not uniform around the E. coli chromosome. Only about 0.4% of the transductants for $sup–159$ have tandem duplications (HILL et al.
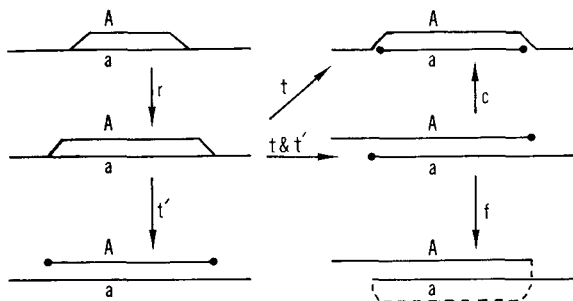
Figure 4.—Dependence of the outcome of integration processes on the mode of replicative step termination. The integration of a fragment (A) into a homologous chromosomal region is initiated as in Figure 3 and replication (r) proceeds. Replication step terminations, in which the branch of a fork continuous with the fragment DNA are broken and conserved, are respectively designated (t') and (t). Temporal simultaneity of termination steps is not assumed. When replication step terminations (t' and t) culminate in chromosome breakage, chromosome continuity can be restored by crossing over (c) or fusion (f).

1969). What factors control the ratio of haploid to heterozygous transductant formation?

Perhaps a controlling factor is the mode of replication step termination in crossing over. Consider an intermediate for fragment integration (Figure 4) formed through two crossovers initiated as depicted in Figure 3. There are three ways in which the intermediate can be disrupted. If the forks continuous with the fragment are both broken, the fragment integration is aborted. If both forks continuous with the corresponding recipient chromosomal segment are broken, the recombinant is haploid. But if alternative forks are broken, the immediate product has genetically overlapping ends (or a terminal repetition since the *E. coli* chromosome is circular). A joining of the ends by legitimate crossing over will again result in the appearance of a haploid recombinant. But as previously noted (Stodolsky, *et al.* 1972) this mode of end joining is competitive with joining through fusion, and the latter process leads to the formation of a tandem duplication. Thus in the context of this scheme, *a genetically local control of the mode of replicative fork disruption* can result in diverse frequencies for the formation of terminally repetitious recombinant intermediates for different chromosomal regions; and the formation of such intermediates for different chromosomal regions; and the formation of such intermediates may culminate in tandem duplication formation. The striking effects of local gene expression on fragment integration described by Drexler (1972) do show that some steps in integration are susceptible to locus-specific control and variation.

The most decisive evidence for the occurrence of recombination-associated replication has been obtained by Stahl and coworkers (1971, 1973) in analyses of crosses between density-labeled bacteriophage lambda. The occurence of such replication provides a basis for explaining certain gene conversion phenomena (Boon and Zinder 1971; Stadler and Towe 1971). The evidence presented herein is novel in character; the participants in crossing over are recovered as

part of a single recombinant structure, the $\Delta^{proA-lac}/lacV$ chromosome, rather than as separated recombinant products.

A duplication phenomenon which may be related to that described herein has been analyzed by HILL, SCHIFFER and BERG (1969). They observed that tandem duplications are constructed with high efficiency in a haploid recipient when the transduced fragment encodes both the selected donor marker and the joint of the donor's duplication. This phenomenon was explained in terms of sequences of legitimate crossovers involving the fragment and two sister recipient chromosomes, through which a segment of one sister chromosome is deleted and utilized in the reconstruction of the duplication. An alternative explanation for this reconstruction phenomenon is as follows. The genes of the duplication which were not cotransduced with the repeat point were added to the fragment in the recipient, through a replication accompanying crossing over. The unit repeat can then be added to the recipient chromosome through purely legitimate crossovers and a companion deletion needn't be produced. BOON and ZINDER (1971) have also commented upon this possibility.

The trick utilized herein to produce the F'episomes in Hfr recipients can undoubtedly be extended to Hfr strains for which F is not adjacent to a deletion. Perform an $a^--b^+ \times a^+-F-b^-$ cross, selecting for transductants with an $A^+ B^+$ phenotype. If a and b are very tightly linked genes in the donor, the contribution of $a^+ b^+$ haploid recombinants to the transductant population will be minimal, and an enrichment for partial diploids will therefore be achieved. Among the heterozygous recombinants, those with a $a^--b^--jnt-a^+-F-b^-$ chromosomes will segregate $a^-b^-/F'a^+b^+$ strains. The technique of heterozygote selection has sufficed for a related task—the isolation of transductants with specialized transducing derivatives of P1 as $a^+b^-(P1da^-b^+)$ lysogens (STODOLSKY 1973b). This study and its predecessor (STODOLSKY, RAE and MULLENBACH 1972) were in fact initiated as control experiments without P1 helper, in a study of the origins of specialized transducing derivatives of bacteriophage P1 (RAE and STODOLSKY 1974).

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